Purification of pharmaceutical drugs by mass-based fraction collection at higher flow rates

Application

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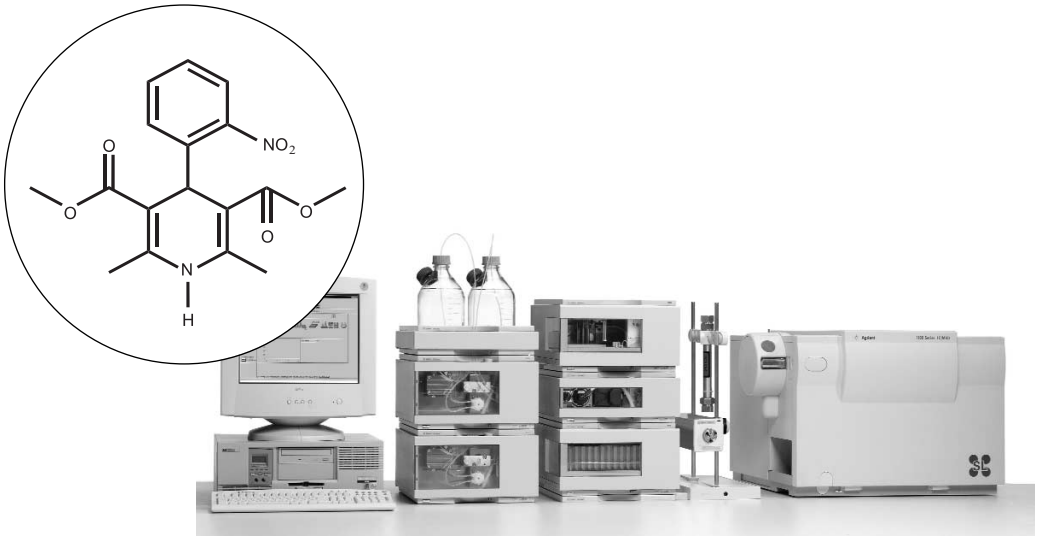

Abstract

Preparative HPLC is probably the most important tool for purification of compounds in pharmaceutical research. The amount of compound to be purified ranges from low milligram amounts in drug discovery to hundreds of grams and a few kilograms during drug development and clinical trials. The Agilent 1100 Series purification system, equipped with a mass-selective detector (MSD), offers flexible solutions for purification from micrograms to grams. In this Application Note we show purification of the three antianginal drugs nifedipin, nisoldipin and nimodipin in the lower milligram range as an example application.

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Introduction

Nifedipin, nimodipin and nisoldipin are antianginal drugs with 1,4-dihydropyridine structure. They are used as calcium antagonists in cases of hypertonia, cardiac disrythmia and angina pectoris. In this Application Note we describe the isolation of the three compounds from a mixture as an example application for purification of pharmaceutical drugs. The goal of the application was to purify 20 mg of each compound in a single run and to get three fractions containing only the desired substances. To achieve this the Agilent 1100 Series purification[1,2] system PS (preparative scale) was equipped with an 1100 Series MSD for mass-based fraction collection.

Equipment

The system used included:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series mass-selective detector (MSD)
- Agilent 1100 Series isocratic pump (as make-up pump)
- Agilent active splitter

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the Purification/ HighThruput software (rev. A.01.01).

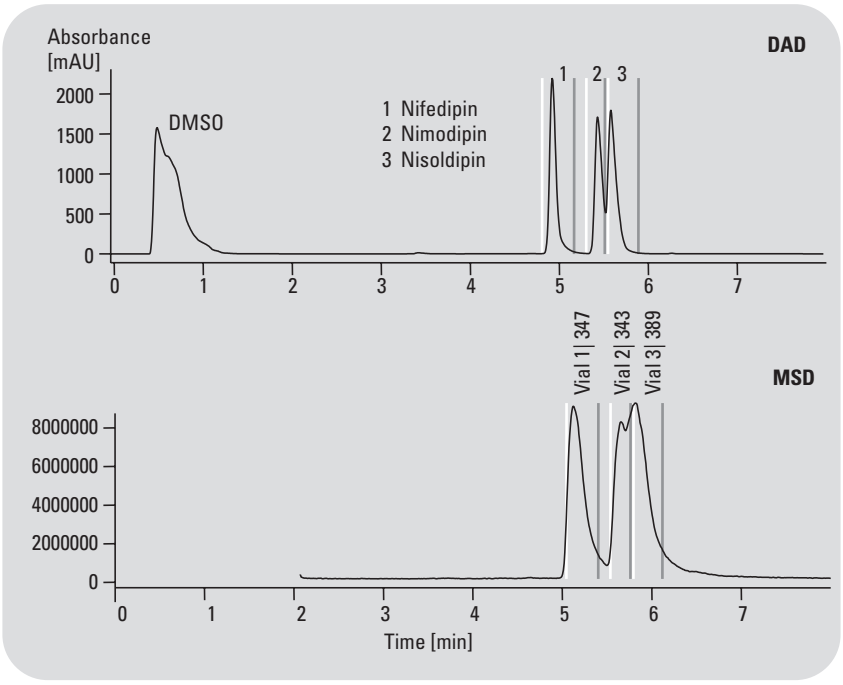

Figure 1
Result of mass-based fraction collection

Results and Discussion

System set-up and configuration

The system was configured to operate a generic method at a flow rate of 25 mL/min on a 21.2 × 50 mm Zorbax SB-C18 column. The flow coming from the column was split after the UV detector – the main flow going to the fraction collector and the split flow going to the MSD. Since the samples were dissolved in DMSO the method was set-up to make sure the DMSO elutes before any compound of interest. Therefore, water/acetonitrile 90:10 was pumped through the column for two minutes before the gradient

Columns	ZORBAX SB-C18
	21.2 × 50 mm, 5 µm
Mobile phases:	A= water + 0.1 % HOAc
	B= acetonitrile +0.1 % HOAc
Gradient:	at 0 min 10 % B
	at 2 min 10 % B
	at 6 min 90 % B
	at 7 min 90 % B
	at 8 min 10 % B
Stop time:	8 min
Post time:	3 min
Flow rate:	25 mL/min
Injection:	500 µL
Column temp.:	ambient
UV detector:	DAD: 220/16nm
	(ref. 360/60 nm), Preparative
	flow cell (0.06-mm pathlength)

MSD

Make-up flow:	1 ml/min
Make-up solvent:	water/acetonitrile 25:75
	+ 0.1 % HOAc
Ionization mode:	API-ES positive
Scan-range m/z:	200–500 starting at 2 min
Fragmentor:	20 Volt
Drying gas flow:	13 l/min
Nebulizer press.:	55 psig
Drying gas temp.:	350 °C
Cap. voltage:	3000 V

was started. To avoid contamination of the MSD with DMSO the stream selection valve was switched to the waste position for the first 2 minutes of the run.

Mass-based fraction collection

The molecular masses of the three compounds are 346.34 for nifedipin, 418.45 for nimodipin and 388.42 for nisoldipin. All three compounds show strong fragmentation in positive ionization mode. Therefore, nimodipin was not triggered on the molecular mass of 418 but on the dominant fragment mass of 342. Figure 1 shows the result of the mass-based fraction collection for triggering on the $[M+H]^+$ - ion.

Re-analysis of fractions

To check the performance of the purification run the collected fractions were re-analyzed on an analytical HPLC system using a method that was calibrated with pure standards before. The chromatograms of the fractions are shown in figure 2. The analytical results are summarized in table 1. The injected amounts of nifedipin, nimodipin and nisoldipin were 19.43 mg, 19.05 mg and 18.92 mg, respectively.

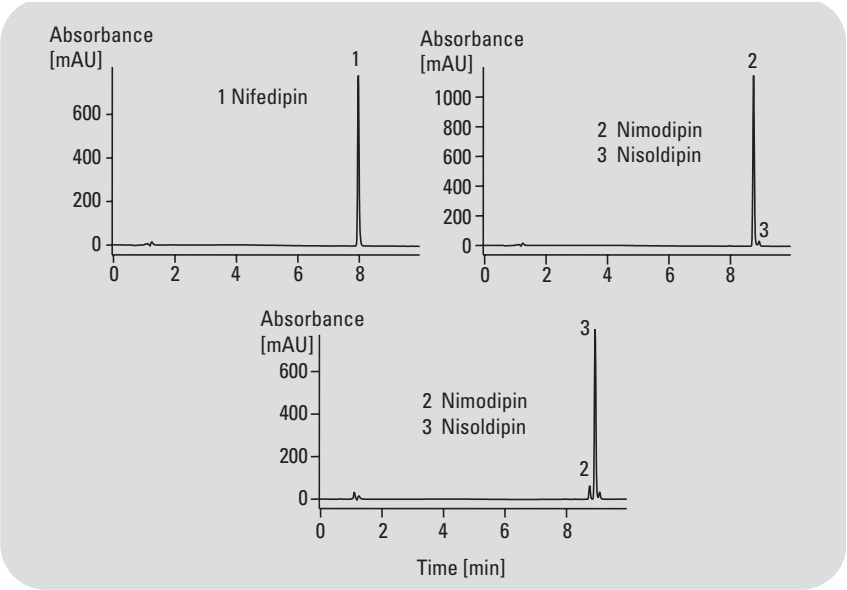

Figure 2
Re-analysis of fractions

	Nifedipin] [mg]	Mimodipin [mg]	Nisoldipin [mg]		
Fraction 1	18.90	0.11	0.16	Purity Nifedipin	98.6 %
Fraction 2	0.29	17.66	0.77	Purity Nimodipin	94.4 %
Fraction 3	0.49	1.66	18.36	Purity Nisoldipin	89.5 %
Recovery [mg]	19.68	19.43	19.29		
Recovery [%]	101.3	102.0	101.9		

Table 1
Results of fraction re-analysis

Conclusion

In this Application Note we showed purification of three antianginal drugs by mass-based fraction collection at a flow rate of 25 mL/min. Even with a highly overloaded column the re-analysis of the collected fractions showed that about 20 mg of each compound could be purified in a single run with high recovery and a purity of 90 % or more. This result proves the excellent performance of the Agilent 1100 Series purification system equipped with MSD at higher flow rates.

References

1.
"New perspectives in purification with HPLC and HPLC/MS", *Agilent Technologies Brochure*, **2001**, publication number 5988-3673EN.

2.
"Agilent 1100 Series Purification Platform — Customized system configurations", *Agilent Technologies Flyer*, **2001**, publication number 5988-3751EN.

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